COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Tom Rapoport of Harvard Medical School, of Harvard University, Boston, Massachusetts, United States of America, declare as follows:

1. I am currently a Professor of Cell Biology for the Howard Hughes Medical Institute in the Department of Cell Biology at the Harvard Medical School in Boston, Massachusetts. In 1995, I was appointed Professor of Cell Biology at Harvard Medical School. Prior to that appointment, I was Group Leader at the Max-Delbruck-Center for Molecular Medicine, Germany, from 1992-1994. From 1985-1992, I was the Group Leader at the Institute for Molecular Biology, Germany. From 1972-1985, I was a Research Assistant at the Zentralinstitut for Molekularbiologie der Akademie der Wissenschaften der DDR.

2. Since the early 1970s, my research has focused on protein processing and intracellular transport and the role of signal sequences in protein translocation across cell membranes, as evidenced by my curriculum vitae, which lists the publications that I have authored or co-authored. My research in the area of protein translocation has encompassed bacterial, yeast and mammalian systems. I am also broadly interested in examples of nonclassical secretion

pathways of mammalian proteins, including, fibroblast growth factor (FGF). Now shown to me and marked "TP-1" is a copy of my curriculum vitae.

3. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to review Australian Patent Application Au-B-696764 (73941/94) in the name of HGS, entitled "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), which claims priority and has a virtually identical specification to U.S. application no. 08/207,550, filed March 8, 1994. I have also been asked to provide my comments and opinions as to what the patent specification would provide or teach to one of ordinary skill in the art of protein processing as of the earliest filing date of the HGS patent specification, March, 1994. For purposes of this analysis, I considered not only what I knew and appreciated at the relevant time, but what was expected to be known by graduate students and postdoctoral fellows who were in my laboratory at the relevant time. I have also been asked to comment on the state of the art of signal sequences and protein processing as of the earliest filing date of the HGS patent specification, March, 1994.

4. By the late 1970s, secreted proteins and the signal sequences required to route these proteins through the cell were well understood. For example, it was known that specific signals, often called signal sequences, were required to direct secreted proteins outside of the cell. These signal sequences were known to be located at the N-terminus of the secreted protein and removed either during or shortly after translocation of the protein across the endoplasmic reticulum and to the outside of the cell.

5. By 1994, it was known that, in mammals, signal sequences were typically located at the beginning of the secreted protein, often comprising 20 amino acids, and were characterized by a stretch of at least six or seven consecutive hydrophobic amino acids, the majority of which are leucine residues. Outside of the consecutive hydrophobic residues, there were other known requirements

for signal sequences, including the type of residues which provide the information to direct the cell to remove the signal sequence, i.e., the cleavage site. This cleavage site was known to contain small aliphatic residues, such as alanines at positions -1 and -3 to the N-terminus of the cleavage site, and was known to be usually found five to seven residues downstream from the stretch of consecutive hydrophobic residues.

6. By 1994, researchers were able to characterize signal sequences by their hydrophobic nature. Such sequences were often identified by an inspection of the amino acid sequence. Alternatively, computer programs, such as P SORT and SIGNAL P were, and still are today, standard tools used to predict the presence or absence of signal sequences. There was however no certainty with such approaches.

7. I have reviewed and analyzed the polynucleotide, and amino acid sequence, identified by HGS to encode the human VEGF-2 protein, as set forth in Figure 1 of the HGS patent specification. The human VEGF-2 protein is described in the HGS patent specification as structurally related to the PDGF/VEGF family of growth factors, a known family of secreted proteins. The HGS patent specification further states that the VEGF-2 polynucleotide is predicted to contain an open reading frame of approximately 350 residues, which encodes VEGF-2. (See, the HGS patent specification at page 5, lines 25-27). The specification reports that at the amino acid level VEGF-2 exhibits the highest homology to vascular endothelial growth factor (30% identity), followed by PDGF alpha (23%) and PDGF beta (22%). (See, the HGS patent specification at page 5, lines 28-31). The VEGF-2 protein was further characterized in the HGS patent specification as containing eight cysteines which are conserved among all four members of the family, and in addition also contains the conserved or signature motif PXCVXXXRCXGCCN which is found in all members of the PDGF/VEGF family. (See, the HGS patent specification at page 5, lines 31-33). The HGS patent specification speculates that the first 24

residues of the 350 amino acid sequence may encode a signal sequence. (See, the HGS patent specification at page 5, lines 26-27).

8. Attached as "Annexure TP-2" is an annotated copy of Figure 1 of the HGS patent specification which sets forth the nucleotide and amino acid sequence of VEGF-2 identified by HGS. The figure is further annotated to indicate specific amino acid residues and those portions of the VEGF-2 sequence which will become relevant throughout this declaration. For consistency of nomenclature, the numbering of amino acid residues will be referred to in the context of what is now known as the 419 amino acid form of VEGF-2. As shown in Annexure TP-2, the 350 amino acid form of VEGF-2 corresponds to amino acid residues 70 to 419 of the 419 form of VEGF-2.

9. By 1994, had I or any one skilled in the art, such as any Ph.D. scientist or candidate in my laboratory, been presented with the HGS patent specification, (see ¶ 7 above) one would recognize that the VEGF-2 protein is a secreted protein. Based on the characterization of the VEGF-2 protein set forth in the HGS patent specification, one would recognize that the protein was a member of the PDGF/VEGF family of growth factors. The PDGF/VEGF family of growth factors, like other growth factors, must be secreted in order to exert their growth promoting or mitogenic effects. Since all previously identified members of the PDGF/VEGF family were known to be secreted, one would expect the newly identified VEGF-2 to also be secreted.

10. Had I or any skilled artisan been presented with the HGS patent specification in 1994 and wanted to express the VEGF-2 protein, one would proceed with inspecting the 350 amino acid sequence set forth in Figure 1 to identify a potential signal sequence. As I have already noted, by 1994, this was routinely achieved by visual inspection or could be achieved with the aid of a computer program. Upon inspection of the N-terminal portion of the 350 amino acid sequence, I did not observe the typical conserved motif of a signal sequence.

In fact, the N-terminal portion of the 350 amino acid sequence is not very hydrophobic and contains many charged residues. However, provided with the strong evidence that the 350 amino acid sequence was a secreted protein based on the teaching and recognition of the HGS patent specification that it is a member of a family of secreted growth factors (see ¶ 7 above), I would not rule out that the 350 amino acid sequence may contain an atypical signal sequence. Atypical signal sequences are not without precedent. For example, as of March 1994, it was known that ovalbumin, a secreted protein, contains an atypical and uncleaved signal sequence that is not immediately obvious on simple inspection.

11. The possibility of additional upstream coding regions from the sequence disclosed in Figure 1 of the HGS patent specification would not have dissuaded me nor do I believe it would have dissuaded my Ph.D. students or post-doctorate students from attempting to express the 350 amino acid sequence. By 1994, it was well established that the PDGF/VEGF family of growth factors were expressed initially as precursor proteins which underwent proteolytic processing resulting in a mature form of the protein. Thus, I would predict VEGF-2 to be expressed in a similar way. The 350 amino acid sequence set forth in Figure 1 contains those conserved motifs which are signature motifs for an active form of the protein belonging to the PDGF/VEGF family. Thus in 1994, I would have predicted the protein encoded by the sequence disclosed in Figure 1, containing motifs characteristic of the PDGF/VEGF family, to be secreted and biologically active.

12. By 1994, in order to ensure secretion of the VEGF-2 sequence disclosed in Figure 1, I would have engineered a heterologous signal sequence upstream from the methionine at position 70. Indeed, this approach is specifically taught in the HGS patent specification (at page 14, lines 6-23). Moreover, it would have involved routine practice in 1994 to select a strong signal sequence, such as that of human growth factor or insulin growth factor, and to

engineer such a construct to achieve expression and secretion of the gene product. Even given the possibility that the 350 amino acid sequence may contain an atypical signal sequence, I would still have utilized a strong signal sequence to ensure efficient secretion of the protein, and it would have been standard practice to do so. The upstream signal sequence would be expected to override any weaker signal sequence that may be present downstream, and none of the segments of amino acids in the 350 amino acid sequence are hydrophobic enough to prevent the secretion of the protein through the endoplasmic reticulum and to the outside of the cell. Thus, I would have fully expected to achieve expression and secretion of the VEGF-2 protein using a heterologous signal sequence.

13. The expectation that engineering a signal sequence upstream of the sequence set forth in Figure 1 would result in the expression and secretion of a biologically active protein as set forth in the HGS patent specification, has in fact been subsequently confirmed by one skilled in the field of VEGF-2, Dr. Kari Alitalo. Drs. Kari Alitalo and Vladimir Joukov are named as co-inventors of U.S. Patent No. 6,130,071, issued October 10, 2000, entitled: "Vascular Endothelial Growth Factor C (VEGF-C) Cys 156 Protein and Uses Thereof" (the "Alitalo Patent"). It is my understanding that VEGF-2 and VEGF-C are terms used to refer to the same molecule. The Alitalo Patent describes several fragments of VEGF-C which were engineered to be expressed and secreted from cells. Cells were engineered to express the VEGF-2 fragments by fusing a signal sequence to the nucleotide sequence encoding the protein fragment. Secreted protein fragments were obtained from the cell culture medium and tested for activity. The working examples of the Alitalo Patent demonstrate that VEGF-C fragments spanning residues 103-419 or 112-419 (see Annexure TP-2) which are fused in frame to a signal sequence are secreted into the culture medium. (Alitalo Patent, column 47, lines 44 to 48). The Alitalo Patent also demonstrates the use of a heterologous signal sequence to ensure secretion of a fragment of VEGF-C. A fragment

spanning residues 104-213 (see Annexure TP-2) was fused in frame to a heterologous signal sequence which resulted in the secretion of a biologically active form of VEGF-C. (Alitalo Patent, column 46, lines 5 to 10). Furthermore, a publication subsequent to the priority date of the HGS patent specification, which Dr. Alitalo co-authored, Joukov et al, 1997, EMBO J. 16:3898-3911 ("Joukov"), describes two VEGF-C mutants in which the native VEGF-C signal sequence was fused in frame with residues 103-419 or residues 103-227. Both VEGF-C mutants were shown to be secreted into the culture medium, (Joukov at page 3901) using experimental procedures that were routine by 1994. Thus, both Alitalo and Joukov confirm that the fusion of a signal sequence in frame to VEGF-2 fragments will result in secreted protein fragments.

14. The expectation that I would have had from reading the HGS patent specification and the expectation that I would have expected others to have had is that the sequence set forth in Figure 1 does indeed contain the conserved motifs which would confer biological activity to the VEGF-2 protein as set forth in the HGS patent specification (see ¶ 7 above) has also been subsequently confirmed by Dr. Alitalo. In the Alitalo Patent and Joukov, the VEGF-2 protein fragments were tested for VEGF-C biological activity in different assays. The Alitalo Patent expressed and assayed several fragments in this manner, including fragments spanning residues 103-225, 103-419, 104-213, and 112-419 (see Annexure TP-2), and as described in ¶ 13, above. Each of the fragments assayed were found to be biologically active. Based on these observations and sequence comparisons of the VEGF family, the Alitalo Patent states that still smaller fragments of the 419 amino acid sequence of VEGF-C will retain biological activity. (Alitalo Patent, column 47, line 57 to column 48, line 2). The Alitalo Patent further states that a protein which retains the conserved motif RCXXCC (e.g., a polypeptide comprising from about residue 161 to about residue 211, see Annexure TP-2) is postulated to

retain biological activity. (Alitalo Patent, column 47, line 57 to column 48, line 2).

15. As observed in the Alitalo Patent and Joukov, VEGF-C fragments spanning residues 103-225, 103-419, 104-213, and 112-419 (see Annexure TP-2) fused in frame with signal sequences contained sufficient information to be processed by the cell to result in a biologically active form of VEGF-C. Furthermore, Alitalo also predicts that a VEGF-C fragment spanning residues 161-211 of VEGF-C (see Annexure TP-2) contains sufficient information to confer biological activity. Thus, if these VEGF-C fragments spanning residues 103-225, 103-419, 104-213, 112-419 and 161-211 contain sufficient information to allow for processing of a biologically active form of VEGF-C, the longer sequence set forth in the HGS patent specification which spans residues 70-419 (see Annexure TP-2), fused in frame to a signal sequence, should also contain sufficient information to be processed by the cell to result in a biologically active form of VEGF-C. According to the Alitalo Patent and Joukov, proteolytic processing of VEGF-C appears to differ depending on the cell used to achieve expression of the VEGF-C protein (Joukov at ¶ spanning pages 3906-3907). However, both Alitalo and Joukov demonstrate that regardless what type of mammalian or yeast cells are used to express VEGF-C, a biologically active VEGF-C protein is obtained. (Joukov at ¶ spanning pages 3906-3907; Alitalo Patent at column 46, lines 5-10). Consequently, regardless of the cell line used to express VEGF-C and the exact proteolytic processing or glycosylation that results, a biologically active fragment of VEGF-C can still be obtained. Thus, consistent with the teaching of the HGS patent specification, residues 70-419 as set forth in Figure 1 also contain sufficient information to confer VEGF-C biological activity.

16. The Alitalo Patent and Joukov have clearly confirmed that the teaching of the HGS patent specification, that the fusion of a signal sequence to a fragment of VEGF-C will result in the successful secretion of that fragment from the cell

into the culture medium. Thus, according to the teaching of the HGS patent specification, residues 70-419 as set forth in Figure 1 fused in frame to a signal sequence will result in its successful secretion from the cell into the culture medium.

17. In sum, as described in the HGS patent specification, Dr. Alitalo has subsequently demonstrated and/or predicted that VEGF-C fragments spanning residues 103-227, 103-419, 104-213, 112-419 and 161-211 (see Annexure TP-2) when fused in frame to a signal sequence result in a secreted gene product which retains VEGF-C biological activity. Thus, the sequence set forth in the HGS patent specification, spanning residues 70 to 419 of VEGF-C (see Annexure TP-2) fused in frame to a signal sequence should also result in a protein which is secreted and retains VEGF-C biological activity.

18. In my opinion, I or one skilled in the art would identify the VEGF-2 protein as a novel member of the PDGF/VEGF family of growth factors, and as such, would recognize that VEGF-2 is also a secreted growth factor, based on the HGS patent specification in combination with the state of the art as of March, 1994. I or one skilled in the art would recognize the utility in using a heterologous signal sequence to achieve expression and secretion of the VEGF-2 protein, based on the HGS patent specification in combination with the state of the art as of March, 1994. Hence, I or one skilled in the art following the teaching of the HGS patent specification coupled with the knowledge of the art at March 1994, would have predicted and expected to achieve expression and secretion of a protein which retains VEGF-2 biological activity and it would have been obvious to carry out those experiments to achieve that purpose.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Tom Rapoport, _____,

at Boston Massachusetts, on this 13th day of ~~November~~ December 2000;

before me: _____.

Notary Public

LORNA L. FARGO
MY COMM. EXPIRES 12-01-06

Curriculum vitae

Name: Tom A. Rapoport

Address: Howard Hughes Medical Institute
Harvard Medical School
Department of Cell Biology
240 Longwood Avenue
Boston, MA 02115-6091

private address:

65 Greenough Street
Brookline, MA 02445

Phone: (617) 432-0637 (office)
(617) 566-6575 (home)

Personal data: born 06/17/47 in Cincinnati (USA)
married, 3 children

Education:

1965 - 1966	High school specialized in mathematics and natural sciences at the Humboldt-University Berlin, graduation with "honors"
1966 - 1972	Study of chemistry and biochemistry at the Humboldt-University Berlin, graduation with "honors"
1972	Ph.D. for work on "The mechanism of the inorganic pyrophosphatase of baker's yeast"
1977	"Habilitation" for work on "The development of a control theory for the mathematical modelling of metabolic pathways"

Areas of research:

1969 - 1972	enzyme kinetics, enzymology, protein purifications

	1970	- 1980	mathematical modelling of metabolic systems, studies on the regulation of the glycolysis in erythrocytes
	1972	- 1980	biosynthesis of carp insulin, cloning of the cDNA for carp insulin, expression of the gene in E.coli, oocyte injection of mRNAs
	1972	-	intracellular protein transport, transport of proteins across the endoplasmic reticulum membrane, membrane biogenesis
Academic positions:	1972	- 1985	Research Assistant at the Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften der DDR
	1985	- 1992	Professor of Cell Biology
	1985	- 1992	Group Leader at the Institute for Molecular Biology
	1992	- 1994	Group Leader at the Max-Delbrück-Center for Molecular Medicine
	1995	- 1997	Professor of Cell Biology at Harvard Medical School
	1997	- present	HHMI Professor of Cell Biology at Harvard Medical School

Memberships: German Biochemical Society
Academy of Sciences
Academea European
EMBO
American Society for Cell Biology

Honors: Johannes-Müller-prize of the Society for Experimental Medicine

Rudolf-Virchow-prize

Sabbatical:	1982	3 month stay in the laboratory of Dr. G. Blobel (Rockefeller University New York)

Major Committee Assignments:

1996-1997	Standing Committee on Promotions, Reappointments and Appointments in the Faculty of Medicine at Harvard Medical School
1996-present	Various promotion committees
1996-present	Various Ph.D. Thesis Committees
1997-1999	Vice Chair/Chair, Gordon Research Conference on Molecular Membrane Biology
1999-present	NIH study section (permanent member)

Editorial Boards:

1980-1988	FEBS Letters
1989-present	EMBO Journal
1989-present	The Journal of Cell Biology
2000	EMBO Reports

Teaching Report:

1996-1998	Co-director, Cell Biology 201 course
1999-2000	Director, Cell Biology 201 course
1998-1999	CBC course (medical students)
1998-1999	Molecular Machines course

List of publications

01. Rapoport, T. A., Höhne, W. E., Reich, J. G., Heitmann, P., and Rapoport, S. M. (1972) Eur. J. Biochem. 26, 237-246

A kinetic model for the action of the inorganic pyrophosphatase from baker's yeast; the activating influence of magnesium ions

02. Höhen, W. E., Rapoport, T. A., and Heitmann, P. (1972) Acta biol. med. germ. 29, 841-850

Eine automatische Apparatur zur Ermittlung der kinetischen Parameter enzymatischer Reaktionen

03. Höhne, W. E. and Rapoport, T. A. (1973) Eur. J. Biochem. 33, 323-331

Slow conformational changes of the inorganic pyrophosphatase from baker's yeast induced by divalent metal ions

04. Rapoport, T. A., Höhne, W. E., Heitmann, P., and Rapoport, S. M. (1973) Eur. J. Biochem. 33, 341-347

Binding of ligands to the inorganic pyrophosphatase of baker's yeast

05. Heinrich, R. and Rapoport, T. A. (1973) Abhandlg. d. AdW der DDR VII. Internat. Symp. über Struktur und Funktion der Erythozyten, S. 21-27

On the theory of enzymatic systems with application to the glycolysis of erythrocutes

06. Heinrich, R. and Rapoport, T. A. (1973) Acta biol. med. germ. 31, 479-494

Linear theory of enzymatic chains; its application for the analysis of the crossover theorem and of the glycolysis of human erythrocytes

07. Heinrich, R. and Rapoport, T. A. (1974) Eur. J. Biochem. 42, 89-95

A linear steady state treatment of enzymatic chains; general properties,control and effector strength

08. Heinrich, R. and Rapoport, T. A. (1974) Eur. J. Biochem. 42, 97-105

A linear steady state treatment of enzymatic chains; critique of the crossover theorem and a general procedure to identify interaction siteswith an effector

09. Rapoport, T. A., Heinrich, R., Jacobasch, G., and Rapoport, S. M. (1974) Eur.J. Biochem. 107-120

A linear steady state treatment of enzymatic chains; a mathematicalmodel of the glycolysis of human erythrocytes

10. Lukowsky, A., Prehn, S., and Rapoport, T. A. (1974) Biochem. Biophys. Acta 359, 248-252

Biosynthesis of proinsulin in islets of Langerhans of carp (Cyprinus carpio)

11. Rapoport, T. A., Prehn, S., Lukowsky, and A., Junghahn, I. (1974) Acta Biol. med. germ. 33, 953-961

Biosynthesis of proinsulin of carp (Cyprinus carpio)

12. Heinrich, R. and Rapoport, T. A. (1974) Symp. Biol. Hung. 18, 173-212

The regulatory principles of the glycolysis of erythrocytes in vivo and in vitro

13. Rapoport, S. M., Rapoport, T. A., and Heinrich, R. (1974) 9th FEBS meeting Budapest, Publ. House Hung. Acad. Sci. p. 195-209

The regulation of glycolysis in erythrocytes

14. Rapoport, T. A. and Heinrich, R. (1975) Bio Systems 7, 120-129

Mathematical analysis of multienzyme systems; I. Modelling of the glycolysis of human erythrocytes

15. Heinrich, R. and Rapoport, T. A. (1975) Bio Systems 7, 130-136

Mathematical analysis of multienzyme systient control

16. Rapoport, T. A., Heinrich, R. and Rapoport, S. M. (1976) Biochem. J. 154, 449-469

The regulatory principles of glycolysis in erythrocytes in vivo and in vitro; a minimal comprehensive model describing steady states and time-dependent processes

17. Rapoport, T. A., Höhne, W. E., Klatt, D., Prehn, S., and Hahn, V. (1976) FEBS Letters 69, 32-36

Evidence for the synthesis of a precursor of carp proinsulin in a cell free translation system

18. Rapoport, T. A. and Heinrich, R. (1977) Mathematische Modell-bildung, Akademie/Verl., Berlin, S. 208-219

Modellierung metabolischer Systeme

19. Rapoport, T. A., Otto, M., and Heinrich, R. (1977) Acta biol. med. germ. 36, 461-468

An extended model of the glycolysis in erythrocytes

20. Schulz, J., Baufeld, A., Hofmann, E., Rapoport, T. A., Heinrich, R., and Rapoport, S. M. (1977) Acta biol. med. germ. 36, 1379-1391

Regulation of anaerobic glycolysis in Ehrlich ascites tumour cells

21. Heinrich, R., Rapoport, S. M., and Rapoport, T. A. (1977) Prog. Biophys. Molec. Biol. 32, 1-82

Metabolic regulation and mathematical models

22. Rapoport, I., Rapoport, T. A., and Rapoport, S. M. (1978) Acta biol. med. germ. 37, 393-401

Analysis of pH-induced changes of the glycolysis of human erythrocytes

23. Rapoport, T. A., Kohnert, K.-D., Knospe, S., Lukowsky, A., Prehn, S., Schäfer, H., Schmidt, S., and Ziegler M. (1978) Acta biol. med. germ. 37, 1153-1160

Preparation and characterization of insulin of carp (cyprinus carpio)

24. Rapoport, T. A., Thiele, B., Prehn, S., Marbaix, G., Cleuter, Y., Hubert, E., and Huez, G. (1978) Eur. J. Biochem. 87, 229-233

Synthesis of Carp proinsulin in Xenopus oocytes

25. Grosse, R., Rapoport, T. A., Malur, J., Fischer, J., and Repke, K. R. H. (1979) Biochim. Biophys. Acta 550, 500-514

Mathematical modelling of ATP, K+ and Na+ interactions with the Na+ - K+) -ATPase occuring under equilibrium conditions

26. Rosenthal, S., Coutelle, Ch., Hahn, V., Liebscher, D. H., and Rapoport, T. A. (1979) Studia biophysica 76, 147-148

Cloning of vertebrate genes in E. coli: perspective for basic research, technical microbiology and medicine/considering own data

27. Rapoport, T. A., Prehn, S., Huth, A., and Tsamaloukas, A. (1980) Physiol. Sci. 13, 135-146

Biosynthesis of proinsulin of carp

28. Rapoport, T. A., Prehn, S., and Tsamaloukas, A. (1980) (Augustyniak, J., ed), Elsevier, North-Holland, pp 215-228

Biological implications of protein-nucleic acid interactions

29. Prehn, S., Tsamaloukas, A, and Rapoport, T. A. (1980) Eur. J. Biochem. 107, 185-195

Demonstration of specific receptors of the rough endoplasmic reticulum membrane for the signal sequence of carp preproinsulin

30. Liebscher, D. H., Coutelle, Ch., Rapoport, T. A., Hahn, V., Rosenthal, S., Prehn, S., and Williamson, R. (1980) Gene 9, 233-246

Cloning of carp preproinsulin cDNA in the bacterial plasmid

31. Rapoport, T. A., Prehn, S., Tsamaloukas, A., Coutelle, Ch., Liebscher, D. H., Huth, A., and Rosenthal, S. (1980) Ann. N. Y. Acad. Sci. 343, 111-132

Biosynthesis of proinsulin of carp (cyprinus carpio) and characterization and cloning of mRNA

32. Heinrich, R. and Rapoport, T. A. (1980) J. Theoret. Biol. 86, 279-313

Mathematical modelling of translation of mRNA in eucaryotes; steady states, time-dependent processes and application to reticulocytes

33. Rapoport, T. A. (1981) Eur. J. Biochem. 115, 665-669

Intracellular compartmentation and secretion of carp proinsulin synthesized in Xenopus oocytes

34. Prehn, S., Nürnberg, P., and Rapoport, T. A. (1981) FEBS Letters 123, 79-84

A receptor for signal segments of secretory proteins in rough endoplasmic reticulum membranes

35. Huth, A. and Rapoport, T. A. (1982) Gen. Comp. Endocrinol. 46, 158-167

Regulation of the biosynthesis of insulin in isolated Brockmann bodies of the carp (cyprinus carpio)

36. Makower, A., Dettmer, R., Rapoport, T. A., Knospe, S., Behlke, J., Prehn, S., Franke, P., Etzold, G., and Rosenthal, S. (1982) Eur. J. Biochem. 122, 339-345

Carp insulin: amino acid sequence, biological activity and structural properties

37. Bendzko, P., Prehn, S., Pfeil, W., and Rapoport, T. A. (1982) Eur. J. Biochem. 123, 121-126

Different modes of membrane interactions of the signal sequence of carp preproinsulin and of the insertion sequence of rabbit cytochrome b_5

38. Bassüner, R., Huth, A., Manteuffel, R., and Rapoport, T. A. (1983) Eur. J. Biochem. 133, 321-326

Secretion of plant storage globulinpolypeptides by Xenopus leavis oocytes

39. Finkelstein, A. V., Bendzko, P., and Rapoport, T. A. (1983) FEBS Letters 161, 176-179

Recognition of signal sequences

40. Hahn, V., Winkler, I., Rapoport, T. A., Liebscher, D. H., Coutelle, Ch., and Rosenthal, S. (1983) Nucl. Acid Res. 11, 4541-4552

Carp pr proinsulin cDNA sequence and evolution of insulin genes

41. Rapoport, T. A., Huth, A., Prösch, S., Bendzko, P., Kääriäinen, L., Bassüner, R., Manteuffel, R., and Finkelstein, A. (1983) in: Protein synthesis (Abraham, A. K. K., Eikhom, T. S., and Pryme, I. F., eds) The Humana Press, Clifton, New Jersey, pp 81-100

Transport of proteins and signal recognition

42. Bassüner, R., Wobus, U., and Rapoport, T. A. (1984) FEBS Letters 166, 314-320

Signal recognition particle triggers the translocation of storage globulin polypeptides from field beans (Vicia faba L.) across mammalian endoplasmic reticulum membrane

43. Huth, A., Rapoport, T. A., and Kääriäinen, L. (1984) EMBO-J. 3, 767-771

Envelope proteins of Semliki Forest virus synthesized in Xenopus oocytes are transported to the cell surface

44. Wiedmann, M., Huth, A., and Rapoport, T. A. (1984) Nature (Lond.) 309, 637-639

Xenopus oocytes can secrete bacterial β-lactamase

45. Pfeiffer, M., Hahn, V., Huth, A., Liebscher, D. H., Pöhlmann, Ch., Rapoport, T.A., Rosenthal, S., Tran-Batcke, A., and Wolf, M. (1984) Biol. Zbl. 103, 637-647

Expression of the DNA sequence coding for carp preproinsulin in E. coli-initiation of translation at the eukaryotic start codon

46. Rapoport, T. A. and Wiedmann, M. (1985) Curr. Tojpics in Membranes and Transport 24, 1-62

Application of the signal hypothesis to the incorporation of integral membrane proteine

47. Rapoport, T. A. (1985) FEBS Letters 187, 1-10

Extensions of the signal hypothesis-sequential insertion model versus amphipathic tunnel hypothesis

48. Rapoport, T. A. (1986) CRC Crit. Rev. Biochem. 20, 73-137

Protein translocation across, and integration into, membranes

49. Kurzchalia, T. V., Wiedmann, M., Girshovich, A. S., Bochkareva, E. S., Bielka, H., and Rapoport, T. A. (1986) Nature 320, 634-636.

The signal sequence of nascent preprolactin interacts with the 54K polypeptide of the signal recognition particle

50. Wiedmann, M., Huth, A., and Rapoport, T. A. (1986) FEBS Letters 194, 139-145

Internally transposed signal sequence of carp preproinsulin retains its function with the signal recognition particle

51. Wiedmann, M., Huth, A., and Rapoport, T. A. (1986) Biochem. Biophys. Res. Commun. 134, 790-796

A signal sequence is required for the functions of the signal recognition particle

52. Wiedmann, M., Kurzchalia, T. V., Bielka, H., and Rapoport, T. A. (1987) J. Cell Biol. 104, 201-208.

Direct probing of the interaction between the signal sequence of nascent preprolactin and the signal recognition particle by specific crosslinking

53. Rapoport, T. A., Heinrich, R., Walter, P., and Schulmeister, Th. (1987) J. Mol. Biol. 195, 621-636.

Mathematical modelling of the effects of the signal recognition particle on translation and translocation of proteins across the endoplasmic reticulum membrane

54. Prehn, S., Wiedmann, M., Rapoport, T. A., and Zwieb, Ch. (1987) EMBO-J. 6, 2093-2097

Protein translocation across wheat germ microsomal membranes requires an SRP-like component

55. Wiedmann, M., Kurzchalia, T. V., Hartmann, E., and Rapoport, T. A. (1987) Nature 328, 830-833

A signal sequence receptor in the endoplasmic reticulum membrane

56. Kurzchalia, T. V., Wiedmann, M., Breter, H., Zimmermann, W., Bauschke, E., and Rapoport, T. A. (1988) Eur. J. Biochem. 172, 663-668

tRNA-mediated labelling of proteins with biotin: a non-radioactive method for the detection of cell-free translation products

57. Bassüner, R., Bäumlein, H., Huth, A., Jung, R., Wobus, U., Rapoport, T. A., Saalbach, G., and Müntz, K. (1988) Plant. Mol. Biol. II, 321-334

Abundant embryonic mRNA in field been (Vicin faba L.) codes for a new class of seed proteins: cDNA cloning and characterization of the primary translation product

58. Wiedmann, M., Wiedmann, B., Voigt, S., Wachter, E., Müller, H.-G., and Rapoport, T. A. (1988) EMBO-J. 7, 1763-1768

Post-translational transport of proteins into microsomal membranes of Candida maltosa

59. Hartmann, E., Wiedmann, M., and Rapoport, T. A. (1989) EMBO-J. 8, 2225-2229

A membrane component of the endoplasmic reticulum that may be essential for protein translocation

60. Huth, A., Petrukhrin, K. E., Modyanov, N. N., and Rapoport, T. A. (1989) Biomed. Biochim. Acta 48, 77-84

SRP-dependent membrane integration of the beta-subunit of Na^+, K^+-ATPase

61. Wiedmann, M., Görlich, P., Hartmann, E., Kurzchalia, T. V., and Rapoport, T. A. (1989) FEBS Lett. 257, 263-268

Photocrosslinking demonstrates procimity of the 34kDa-membrane protein to different portions of preprolactin during translocation through the endoplasmic reticulum

62. Rapoport, T. A., Wiedmann, M., Kurzchalia, T. V., and Hartmann, E. (1989) Biochem. Trans. 17, 325-328

Signal recognition in protein translocation across the endoplasmic reticulum membrane

63. Bernstein, H. D., Rapoport, T. A., and Walter, P. (1989) Cell 58, 1017-1019

Cytosolic protein translocation factors: Is SRP still unique?

64. Hartmann, E., Rapoport, T. A., and Lodish, H. F. (1989) Proc. Natl. Acad. Sci. USA 86, 5786-5790

Predicting the orientation of eukaryotic membrane-spanning proteins

65. Prehn, S., Herz, J., Hartmann, E., Kurzchalia, T. V., Frank, R., Römisch, K., Dobberstein, B., and Rapoport, T. A. (1990) Eur. J. Biochem. 188, 439-445

Structure and biosynthesis of the signal sequence receptor

66. Müsch, A., Hartmann, E., Rohde, K., Rubartelli, A., Sitia, R., and Rapoport, T. A. (1990) Trend Biochem. Sci. 15, 86-88

A novel pathway for secretory proteins?

67. Rapoport, T. A. (1990) Trends Biochem. Sci. 15, 355-358

Protein transport across the ER membrane

68. Görlich, D., Prehn, S., Hartmann, E., Herz, J., Otto, A., Kraft, R., Wiedmann, M., Knespel, S., Dobberstein, B., and Rapoport, T. A. (1990) J. Cell Biol. 111, 2283-2294

The signal sequence receptor has a second subunit and is part of a translocation complex in the endoplasmic reticulum as probed by bifunctional reagents

69. Vogel, F., Hartmann, E., Görlich, D., and Rapoport, T. A. (1990) Eur. J. Cell Biol. 53, 197-202

Segregation of the signal sequence receptor protein in the rough endoplasmic reticulum membrane

70. Rapoport, T. A. (1991) Nature 349, 107-108

A bacterium catches up

71. Rapoport, T. A. (1991) FASEB-Journal 5, 2792-2798

Protein transport across the endoplasmic reticulum membrane: facts, models, mysteries

72. Görlich, D., Kurzchalia, T. V., Wiedmann, M., and Rapoport, T. A. (1991) Meth. Cell Biol. 34, 241-261

Probing the molecular environment of translocating polypeptide chains by crosslinking

73. High, S., Görlich, D., Wiedmann, M., Rapoport, T. A., and Dobberstein, B. (1991) J. Cell Biol. 113, 35-44

The identification of proteins in the proximity of signal-anchor sequences during their targeting to and insertion into the membrane of the ER

74. Rapoport, T. A., Görlich, D., Müsch, A., Hartmann, E., Prehn, S., Wiedmann, M., Otto, A., Kostka, S., and Kraft, R. (1991) Antonie van Leeuwenhoek 61, 119-122

Components and mechanism of protein translocation across the ER membrane

75. Müsch, A., Wiedmann, M., and Rapoport, T. A. (1992) Cell, Vol. 69, 343-352

Yeast Sec-proteins interact with polypeptides traversing the endoplasmic reticulum membrane

76. Görlich, D., Hartmann, E., Prehn, S., and Rapoport, T. A. (1992) Nature, Vol. 357, 47-52

A protein of the endoplasmic reticulum involved early in polypeptide translocation

77. Hartmann, E., Rapoport, T. A., and Prehn, S. (1992) Nature 358, 198.

Signal sequence identified

78. Görlich, D., Prehn, S., Hartmann, E., Kalies, K.-U., and Rapoport, T.A. (1992) Cell 71, 489-503

A mammalian homolog of Sec61p and SecYp is associated with ribosomes and nascent polypeptides during translocation

79. Rapoport, T. A. (1992) Science 258, 931-936

Transport of proteins across the endoplasmic reticulum (ER) membrane

80. Hartmann, E. and Rapoport, T.A. (1992) in: Membrane Biogenesis and Protein Targeting (Neupert, W. and Lill, R., Eds.; Elsevier) 119-127

Translocation of proteins through the endoplasmic reticulum membrane: investigation of their molecular environment by cross-linking

81. Kutay, U., Hartmann, E. and Rapoport, T.A. (1993) Trends in Cell Biology 3, 72-75

A class of membrane proteins with a C-terminal anchor

82. Hartmann, E., Görlich, D., Kostka, S., Otto, A., Kraft, R., Knespel, S., Bürger, E., Rapoport, T.A. and Prehn, S. (1993) Eur. J. Biochem. 214, 375-381

A tetrameric complex of membrane proteins in the endoplasmic reticulum

83. High, St., Andersen, S.S.L., Görlich, D., Hartmann, E., Prehn, S., Rapoport, T.A. and Dobberstein, B. (1993) The Journal of Cell Biology 121, 4, 743-750

Sec61p is Adjacent to Nascent Type I and Type II Signal-Anchor Proteins during Their Membrane Insertion

84. Görlich, D. and Rapoport, T.A. (1993) Cell 75, 615-630

Protein Translocation into Proteoliposomes Reconstituted from Purified Components of the ER membrane

85. Jungnickel, B. and Rapoport, T.A. (1994) FEBS Letters 329, 3, 268-272

DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid) inhibits an early step of protein translocation across the mammalian ER membrane

86. Hartmann, E., Sommer, Th., Prehn, S., Görlich, D., Jentsch, St. and Rapoport, T.A. (1994) Nature 367, 654-657

Evolutionary conservation of components of the protein translocation complex

87. Kalies, K.-U., Görlich, D., and Rapoport, T.A. (1994) The Journal of Cell Biology 126, 925-934

Binding of ribosomes to the rough endoplasmic reticulum-mediated by the Sec61p-Complex

88. Mothes, W., Prehn, S., and Rapoport, T. A. (1994) EMBO-J. 13, No. 17, 3973-3982

Systematic probing of the environment of a translocating secretory protein during translocation through the ER membrane

89. Jungnickel, B., Rapoport, T.A., and Hartmann, E. (1994) FEBS Letters 346, 73-77

Protein translocation: common themes from bacteria to man

90. Kutay, U., Ahnert-Hilger, G., Hartmann, E., Wiedenmann, B., and Rapoport, T. A. (1994) EMBO-J. 14, No. 2, 217-223

Transport route for synaptobrevin via a novel pathway of insertion into the ER membrane

91. Oliver, J., Jungnickel, B., Görlich, D, Rapoport, T. A., and High, Stephen (1995) FEBS Letters 362, 126-130

The Sec61 complex is essential for the insertion of proteins into the membrane of the endoplasmic reticulum

92. Panzner, S., Dreier, L., Hartmann, E., Kostka, S., and Rapoport, T. A. (1995) Cell 81, 561-570

Posttranslational protein transport in yeast reconstituted with a purified complex of sec proteins and Kar2p

93. Jungnickel, B., and Rapoport, T.A. (1995) Cell 82, 261-270

A posttargeting signal sequence recognition event in the endoplasmic reticulum membrane

94. Panzner, S., Dreier, L., Hartmann, E., Kostka, S. and Rapoport, T.A. (1995) Cold Spring Harbor Symposia on Quantitative Biology, LX, 31-40

Posttranslational protein transport into the endoplasmic reticulum

95. Lyko, F., Martoglio, B., Jungnickel, B., Rapoport, T.A., and Dobberstein, B. (1995) J. Biol. Chem. 270, 19873-19878.

Signal sequence processing in rough microsomes.

96. Finke, K., Plath, K., Panzner, S., Prehn, S., Rapoport, T.A., Hartmann, E., and Sommer, T. (1996) EMBO-J. 15, 1482-1494

A second trimeric complex containing homologs of the Sec61p- complex functions in protein transport across the ER membrane of S. cerevisiae

97. Rapoport, T.A., Jungnickel, B., and Kutay, U. (1996) Annu. Rev. Biochem. 65, 271-303

Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes

98. Ahnert-Hilger, G., Kutay, U., Chahoud, I., Rapoport, T.A., and Wiedenmann, B. (1996) Eur. Jour. of Cell Biology 70, 1-11

Synaptobrevin is essential for secretion but not for the development of synaptic processes

99. Whitley, P., Grahn, E., Kutay, U., Rapoport, T.A., and von Heijne, G. (1996) J. Biol. Chem. 271, 7583-7586.

A 12-residue-long polyleucine tail is sufficient to anchor synaptobrevin to the endoplasmic reticulum membrane.

100. Bacher, G., Lütcke, H., Jungnickel, B., Rapoport, T.A., and Dobberstein, B. (1996) Nature 381, 248-251

Regulation by the ribosome of the GTPase of the signal-recognition particle during protein targeting

101. Voigt, S., Jungnickel, B., Hartmann, E., and Rapoport, T.A. (1996) Journal of Cell Biology 134, 25-35

Signal sequence-dependent function of the TRAM protein during early phases of protein transport across the endoplasmic reticulum membrane

102. Rapoport, T.A., Rolls, M.M., and Jungnickel, B. (1996) Current Opinion in Cell Biology 8, 499-504

Approaching the mechanism of protein transport across the ER membrane

103. Hanein, D., Matlack, K.E.S., Jungnickel, B., Plath, K., Kalies, K.-U., Miller, K., Rapoport, T.A., and Akey, C. (1996) Cell 87, 721-732

Oligomeric rings of the Sec61p complex induced by ligands required for protein translocation

104. Wiertz, E.J.H.J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T.R., Rapoport, T.A., and Ploegh, H.L. (1996) Nature 384, 432-438

Cytosolic destruction of a type I membrane protein by transfer from the ER to the proteasome involves the Sec61p complex

105. Mothes, W., Heinrich, S., Graf, R., Nilsson, I., von Heijne, G., Brunner, J., and Rapoport, T.A. (1997) Cell 89, 523-533.

Molecular mechanism of membrane protein integration into the endoplasmic reticulum

106. Matlack, K.E.S., Plath, K., Misselwitz, B., and Rapoport, T.A. (1997) Science 277, 938-941

Protein transport by purified yeast Sec complex and Kar2p without membranes

107. Neuhof, A., Rolls, M.M., Jungnickel, B., Kalies, K.-U., and Rapoport, T.A. (1998) Mol. Biol. Cell 9, 103-115

Binding of signal recognition particle gives ribosome/nascent chain complexes a competitive advantage in endoplasmic reticulum membrane interaction

108. Matlack, K.E.S., Mothes, W., and Rapoport, T.A. (1998) Cell 92, 381-390

Protein translocation: tunnel vision

109. Hegde, R.S., Voigt, S., Rapoport, T.A., and Lingappa, V.R. (1998) Cell 92, 621-631

TRAM regulates the exposure of nascent secretory proteins to the cytosol during translocation into the endoplasmic reticulum

110. Mothes, W., Jungnickel, B., Brunner, Josef, and Rapoport, T.A. (1998) J. Cell Biol. 142, 355-364.

Signal sequence recognition in cotranslational translocation by protein components of the endoplasmic reticulum membrane

111. Kalies, K.-U., Rapoport, T.A., and Hartmann, E. (1998) J. Cell Biol. 141, 887-894.

The β subunit of the Sec61 complex facilitates cotranslational protein transport and interacts with the signal peptidase during translocation

112. Plath, K., Mothes, W., Wilkinson, B.M., Stirling, C.J., and Rapoport, T.A., (1998) Cell 94, 795-807.

Signal sequence recognition in posttranslational protein transport across the yeast ER membrane

113. Verhey, K., Lizotte, D.L., Abramson, T., Barenboim, L., Schnapp, B.J., and Rapoport, T.A. (1998) J. Cell Biol. 143, 1053-1066.

Light chain-dependent regulation of kinesin's interaction with microtubules

114. Misselwitz, B., Staeck, O., and Rapoport, T.A. (1998) Molecular Cell 2, 593-603.

J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences

115. Meyer, T.H., Menetret, J.-F., Breitling, R., Miller, K.R., Akey, C.W., and Rapoport, T.A. (1999) J. Mol. Biol. 285, 1789-1800.

The bacterial SecY/E translocation complex forms channel-like structures similar to those of the eukaryotic Sec61p complex

116. Matlack, K.E.S., Misselwitz, B., Plath, K., and Rapoport, T.A. (1999) Cell 97, 553-564.

BiP acts as a molecular ratchet during posttranslational transport of prepro-α-factor across the ER membrane

117. Misselwitz, B., Staeck, O., Matlack, K.E.S., and Rapoport, T.A. (1999) J. Biol. Chem. 274, 20110-20115.

Interaction of BiP with the J-domain of the Sec63p component of the ER protein translocation complex

118. Rolls, M.M., Stein, P.A., Taylor, S.S., Ha, E., McKeon, F., and Rapoport, T.A. (1999) J. Cell Biol. 146, 29-43.

A visual screen of a GFP-fusion library identifies a new type of nuclear envelope membrane protein

119. Shamu, C.E., Story, C.M., Rapoport, T.A., and Ploegh, H.L. (1999) J. Cell Biol. 147, 45-57.

The pathway of US11-dependent degradation of MHC class I heavy chains involves a ubiquitin-conjugated intermediate

120. Rapoport, T.A., Matlack, K.E., Plath, K., Misselwitz, B. and Staeck, O. (1999) Biol. Chem. 380, 1143-50.

Posttranslational protein translocation across the membrane of the endoplasmic reticulum

121. Schroder, K., Martoglio B., Hofmann, M., Holscher, C., Hartmann, E., Prehn, S., Rapoport, T.A. and Dobberstein, B. (1999) EMBO J. 18, 4804-15.

Control of glycosylation of MHC class II-associated invariant chain by translocon-associated RAMP4

122. Dreier, L. and Rapoport, T.A. (2000) J. Cell. Biol. 148, 883-898.

In vitro formation of the endoplasmic reticulum occurs independently of microtubules by a controlled fusion reaction

123. Plath, K. and Rapoport, T.A. (2000), J. Cell Biol. 151, 167-178.

Spontaneous release of cytosolic proteins from posttranslational substrates before their transport into the endoplasmic reticulum

124. Prinz, W.A., Grzyb, L., Veenhuis, M., Kahana, J.A., Silver, P.A., and Rapoport, T.A. (2000) J. Cell Biol. 150, 461-474.

Mutants affecting the structure of the cortical endoplasmic reticulum in S. cerevisiae

125. Heinrich, S.U., Mothes, W., Brunner, J., and Rapoport, T.A. (2000) Cell 102, 233-244.

The Sec61p complex mediates the integration of a membrane protein by allowing lipid partitioning of the transmembrane domain

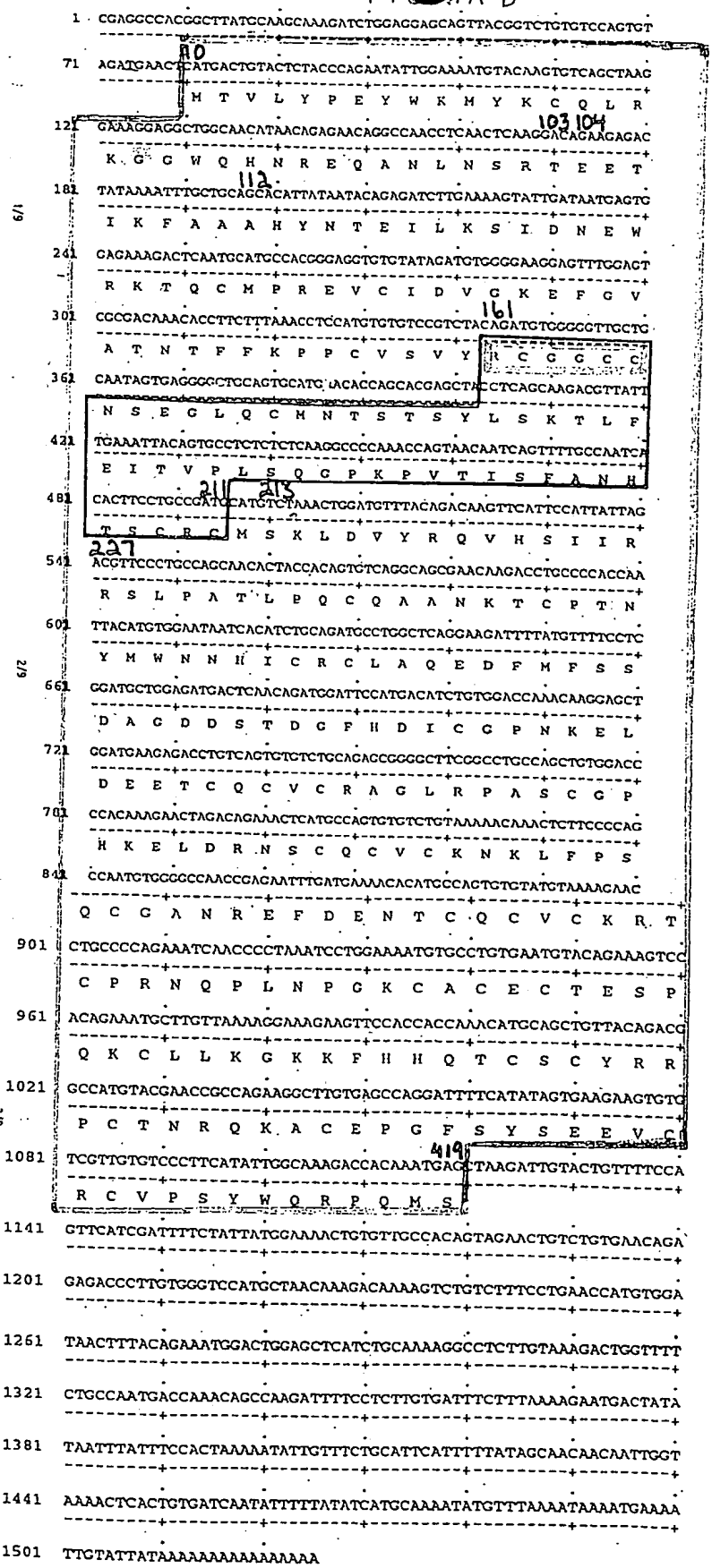
126. Morgan, D.G., Menetret, J.F., Radermacher, M., Neuhof, A., Akey, I.V., Rapoport, T.A. and Akey, C.W. (2000) J. Mol. Biol. 301, 301-321.

A comparison of the yeast and rabbit 80 S ribosome reveals the topology of the nascent chain exit tunnel, inter-subunit bridges and mammalian rRNA expansion segments

127. Prinz, A., Behrens, C., Rapoport, T.A., Hartmann, E. and Kalies, K.U. (2000) EMBO J. 19, 1900-6.

Evolutionarily conserved binding of ribosomes to the translocation channel via the large ribosomal RNA

FIGURE 1A-D



HGS VEGF-2 350 amino acid sequence (70-419)

Minimum sequence required to maintain VEGF-C biological activity as identified by Alitalo

RCXXCC conserved motif

Alitalo VEGF-C fragments:
103-227
103-419
104-213
112-419

ANNEXURE TP-2